

What is claimed is:

1. A method for modifying an oligonucleotide, said method comprising incubating said oligonucleotide with a polynucleotide and a 5'-nuclease wherein at least a portion of said oligonucleotide is reversibly hybridized to said polynucleotide under isothermal conditions and wherein said oligonucleotide is cleaved to provide (i) a first fragment that is substantially non-hybridizable to said polynucleotide and includes no more than one nucleotide from the 5'-end of said portion and (ii) a second fragment that is 3' of said first fragment with reference to the intact oligonucleotide and is substantially hybridizable to said polynucleotide.
2. The method of Claim 1 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of said polynucleotide.
3. The method of Claim 1 wherein a second oligonucleotide is present during said incubating, said second oligonucleotide having the characteristic of hybridizing to a site on said polynucleotide that is 3' of the site at which said oligonucleotide is reversibly hybridized and of being substantially non-reversibly hybridized to said polynucleotide under said isothermal conditions.

4. The method of Claim 3 wherein said second oligonucleotide hybridizes to said polynucleotide at a site contiguous with the site on said polynucleotide at which said first oligonucleotide reversibly hybridizes.

5 5. The method of Claim 4 wherein the amounts of fragments that are formed are at least 100-fold larger than the amount of said polynucleotide.

6. The method of Claim 1 wherein a single nucleoside triphosphate is present during said incubating.

10 7. A method for detecting a polynucleotide analyte, which comprises:

(a) reversibly hybridizing an oligonucleotide with a polynucleotide analyte and a 5'-nuclease under isothermal conditions wherein said polynucleotide analyte serves as a
15 recognition element to enable said 5'-nuclease to cleave said oligonucleotide to provide (i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte and (ii) a second fragment that lies 3' of said first fragment in the intact oligonucleotide and is substantially hybridizable
20 to said polynucleotide analyte wherein at least a 100-fold molar excess of said first fragment and/or said second fragment are obtained relative to the molar amount of said polynucleotide analyte, and

(b) detecting the presence of said first fragment and/or
25 said second fragment, the presence thereof indicating the

presence of said polynucleotide analyte.

8. The method of Claim 7 wherein at least one of said first fragment and said second fragment has a label.

9. The method of Claim 7 wherein said first fragment
5 includes no more than 1 nucleotide from the 5'-end of that portion of said oligonucleotide that hybridizes to said polynucleotide analyte.

10. The method of Claim 7 wherein a second oligonucleotide is present during said reversible hybridizing, said second
10 oligonucleotide having the characteristic of hybridizing to a site on said polynucleotide analyte that is 3' of the site at which said oligonucleotide hybridizes wherein said polynucleotide analyte is substantially fully hybridized to said second oligonucleotide under said isothermal conditions.

11. The method of Claim 8 wherein said oligonucleotide
15 hybridization sites are contiguous.

12. The method of Claim 7 wherein a single nucleoside triphosphate is present during said reversible hybridizing.

13. A method for detecting a polynucleotide analyte, said
20 method comprising:

(a) providing in combination a medium suspected of containing said polynucleotide analyte, a molar excess, relative to the suspected concentration of said polynucleotide analyte, of a first oligonucleotide at least a portion of which is
25 capable of reversibly hybridizing with said polynucleotide

analyte under isothermal conditions, a 5'-nuclease, and a second oligonucleotide having the characteristic of hybridizing to a site on said polynucleotide analyte that is 3' of the site at which said first oligonucleotide hybridizes wherein said
5 polynucleotide analyte is substantially fully hybridized to said second oligonucleotide under said isothermal conditions,

(b) reversibly hybridizing under said isothermal conditions said polynucleotide analyte and said first oligonucleotide, wherein said first oligonucleotide is cleaved
10 as a function of the presence of said polynucleotide analyte to provide, in at least a 100-fold molar excess of said polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte and/or (ii) a second fragment that is 3' of said first fragment
15 in said first oligonucleotide and is substantially hybridizable to said polynucleotide analyte, and

(c) detecting the presence of said first fragment and/or said second fragment, the presence thereof indicating the presence of said polynucleotide analyte.

20 14. The method of Claim 13 wherein said first fragment and/or said second fragment has a label.

15 15. The method of Claim 14 wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.
25

16. The method of Claim 13 wherein said polynucleotide analyte is DNA.

17. The method of Claim 13 wherein said first fragment includes no more than 1 nucleotide from the 5'-end of that
5 portion of said first oligonucleotide that is capable of hybridizing to said polynucleotide analyte.

18. The method of Claim 13 wherein said second oligonucleotide hybridizes to said polynucleotide at a site contiguous with the site on said polynucleotide at which said
10 first oligonucleotide hybridizes.

19. The method of Claim 13 wherein a single nucleoside triphosphate is present in said combination during said reversible hybridizing.

20. A method for detecting a DNA analyte, said method
15 comprising:

(a) providing in combination a medium suspected of containing said DNA analyte, a first oligonucleotide at least a portion of which is capable of reversibly hybridizing with said DNA analyte under isothermal conditions, a 5'-nuclease, and a
20 second oligonucleotide having the characteristic of hybridizing to a site on said DNA analyte that is 3' of the site at which said first oligonucleotide hybridizes wherein said DNA analyte is substantially fully hybridized to said second oligonucleotide under said isothermal conditions,

25 (b) reversibly hybridizing said polynucleotide analyte and

5 said first oligonucleotide under said isothermal conditions, wherein said first oligonucleotide is cleaved to (i) a first fragment that is substantially non-hybridizable to said DNA analyte and (ii) a second fragment that is 3' of said first fragment in said first oligonucleotide and is substantially hybridizable to said DNA analyte wherein at least a 100-fold molar excess, relative to said DNA analyte, of said first fragment and/or said second fragment is produced and

(c) detecting the presence of said first fragment and/or
10 said second fragment, the presence thereof indicating the presence of said DNA analyte.

21. The method of Claim 20 wherein said first oligonucleotide has a substituent that facilitates separation of said first fragment or said second fragment from said medium.

15 22. The method of Claim 20 wherein first fragment and/or said second fragment has a label.

23. The method of Claim 22 wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes,
20 enzyme substrates, radioactive groups and suspendible particles.

24. The method of Claim 20 wherein a single nucleoside triphosphate is present in said combination during said reversible hybridizing.

25 25. The method of Claim 20 wherein said second oligonucleotide hybridizes to said polynucleotide at a site

contiguous with the site on said polynucleotide at which said first oligonucleotide hybridizes.

26. The method of Claim 20 wherein said first oligonucleotide and/or said second oligonucleotide is DNA.

5 ~~27~~ A method for detecting a polynucleotide analyte, said method comprising:

(a) providing in combination a medium suspected of containing said polynucleotide analyte, a first DNA oligonucleotide at least a portion of which is capable of
10 reversibly hybridizing with said polynucleotide analyte under isothermal conditions, a 5'-nuclease, and a second DNA oligonucleotide having the characteristic of hybridizing to a site on said polynucleotide analyte that is 3' of, and contiguous with, the site at which said first DNA
15 oligonucleotide hybridizes wherein said polynucleotide analyte is substantially fully hybridized to said second DNA oligonucleotide under said isothermal conditions,

(b) reversibly hybridizing under said isothermal conditions said polynucleotide analyte and said first DNA
20 oligonucleotide, wherein said first DNA oligonucleotide is cleaved as a function of the presence of said polynucleotide analyte to provide, in at least a 100-fold molar excess of said polynucleotide analyte, (i) a first fragment that is substantially non-hybridizable to said polynucleotide analyte
25 and/or (ii) a second fragment that is 3' of said first fragment

in said first DNA oligonucleotide and is substantially hybridizable to said polynucleotide analyte, and

(c) detecting the presence of said first fragment and/or said second fragment, the presence thereof indicating the presence of said polynucleotide analyte.

28. The method of Claim 27 wherein said first fragment and/or said second fragment has a label.

29. The method of Claim 28 wherein said label is selected from the group consisting of a member of a specific binding pair, dyes, fluorescent molecules, chemiluminescers, coenzymes, enzyme substrates, radioactive groups and suspendible particles.

30. The method of Claim 27 wherein said polynucleotide analyte is DNA.

31. The method of Claim 27 wherein a single nucleoside triphosphate is present in said combination during said reversible hybridizing.

32. A kit for detection of a polynucleotide comprising in packaged combination:

(a) a first oligonucleotide having the characteristic that, when reversibly hybridized under isothermal conditions to at least a portion of said polynucleotide, it is degraded by a 5'-nuclease to provide (i) a first fragment that is substantially non-hybridizable to said polynucleotide and (ii) a second fragment that is 3' of said first fragment in said first

25

oligonucleotide and is substantially hybridizable to said polynucleotide

(b) a second oligonucleotide having the characteristic of hybridizing to a site on said polynucleotide that is separated by no more than one nucleotide from the 3'-end of the site at which said first oligonucleotide hybridizes wherein said polynucleotide is substantially fully hybridized to said second oligonucleotide under said isothermal conditions, and

10 (c) a 5'-nuclease.

33. The kit of Claim 32 which comprises a single nucleoside triphosphate.

34. The kit of Claim 32 wherein said first oligonucleotide and said second oligonucleotide are DNA.

15

* * * * *

Add
A1